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Effect of pretreatment of *Cassia fistula* Linn. leaf extract against subacute CCl_4 induced hepatotoxicity in rats

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CCl_4 alone treatment (0.1ml of liquid paraffin/100g body weight, ip) for 7 days followed by 0.1ml of CCl_4 (in liquid parafiin/100g body weight, ip) from day 8 till day 14, caused a 16 fold increase in lipid peroxidation and a 50% reduction in catalase and glutathione reductase in liver tissue of rats accompanied by an increase in the activities of transaminases, alkaline phosphatase, lactate dehydrogenase and γ - glutamyl transpeptidase in serum as compared to liquid paraffin treated control. Pretreatment of ethanolic leaf extract of *C. fistula* (500mg/kg body weight/day for 7 days) followed by CCl_4 treatment (0.1ml/100g body weight from day 8 till day 14) completely reversed back lipid peroxidation and the activities of catalase and glutathione reductase in the liver tissue towards normalcy. This treatment also reversed the elevated levels of the enzymes in the serum. Ethanolic leaf extract alone treatment did not produce any change in all the parameters studied. The results suggest antioxidant and hepatoprotective properties of *C. fistula* during its pretreatment against CCl_4 induced hepatotoxicity.

Keywords: Antioxidants, *Cassia fistula* L, CCl_4, Hepatotoxicity, Hepatoprotective

Cassia fistula Linn. (Family: Caesalpinaceae) is a medium sized deciduous tree, widely cultivated throughout India as an ornamental plant. Various parts of the plant are used for the treatment of several ailments, the leaves are used as laxative, anti-periodic and in rheumatism[1]. Though leaves and pods are reported to be used in the treatment of jaundice by urban people of North Eastern India[2], the antioxidant and hepatoprotective properties of leaf extract are not well established. Free radical generation and lipid peroxidation of hepatocellular membrane are often implicated as positive factors for the onset of carbon tetrachloride (CCl_4) induced hepatocellular damage[3,4]. Antioxidants play a crucial role in hepatoprotective ability and hence, search for crude drugs of plant origin with this property has become a central focus of studies of hepatoprotection today[5,6].

Though hepatoprotective activity of leaf extract of *C. fistula* is reported[2], the antioxidant properties of the leaf extract against hepatocellular damage needs to be substantiated. Hence, the present study has been undertaken to investigate the antioxidant and hepatoprotective activity of ethanolic leaf extract of *C. fistula* against subacute CCl_4 induced hepatotoxicity in rats.

Materials and Methods

The leaves of *Cassia fistula* Linn. were collected from Tamil Nadu Medicinal Plants Farm and Herbal Concentrates Ltd. (TAMPCOL), Chennai, during July and August. The plant was authenticated by Dr. Narayanappa, Chief Botanist, TAMPCOL and a voucher specimen of this plant is deposited in the Department of Botany, Presidency College, Chennai (Herbarium No: 507).

The leaves were washed, shade dried and powdered coarsely by hand. The particle size of the powdered leaf ranged between 0.5-1 cm. About 100g dry weight of the powdered leaf was soaked in 1 liter of 90% redistilled ethanol for 1 month, as it is an ideal medium for the extraction of both polar and non-polar active principles. Extraction of active principles was allowed to undergo by natural percolation under occasional shaking by swirling movement of the container for about 20-30 times every day at an interval of approximately 7-8 hr. The ethanolic leaf extract was filtered using Whatmann No: 1 filter paper and the filtrate was evaporated to dryness at

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60°C. About 19-20g of crude extract was obtained after evaporation, which corresponds to 19-20% of 100g of dried leaf. The qualitative phytochemical screening of the ethanolic leaf extract was performed[7] and the plant extract showed positive for the presence of alkaloids, glycosides, flavonoids, saponins and tannins.

After getting approval from the Institutional Animal Ethical Committee, Wistar Albino rats of either sex weighing between 180-200g obtained from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, were housed in polypropylene cages and acclimatized for 10 days and were fed pellet diet and water *ad libitum*.

Rats were divided at random into 4 groups of 6 animals each. Group I (normal control) received liquid paraffin (0.1ml/100g body weight, ip), daily for 14 days. Group II (CCl_4 control) received liquid paraffin (0.1ml/100g body weight, ip), daily for 7 days and from 8th day, it was followed by treatment with CCl_4 in liquid paraffin (1:1; 0.2ml/100g body weight, ip), upto 14th day. Group III (pretreatment group; experimental group) animals were pretreated with 500mg/kg body weight of ethanolic leaf extract of *C. fistula* orally (as a suspension in distilled water) from day 1 till day 7 and from 8th day they were treated with CCl_4 in liquid paraffin (1:1; 0.2ml/100g body weight, ip), up to 14 days. Group IV (control for leaf extract alone treatment) animals received ethanolic leaf extract (500mg/kg body weight, orally) from day 1 till day 7 and was followed by liquid paraffin from day 8 till day 14.

Animals were sacrificed 24 hr after last injection. Blood collected into clean tubes from retro orbital plexus of ether anaesthetized rats was allowed to clot and serum separated. The liver was dissected out after decapitation of rats and 1% liver homogenate was prepared in tris-HCl buffer (0.1*M*; *p*H 7.4), which was used for all biochemical assay. Serum alanine transaminase (ALT)[8], aspartate transaminase (AST)[8], alkaline phosphatase (ALP)[9], lactate dehydrogenase (LDH)[9] and gamma glutamyl transpeptidase (γ-GT)[10] were measured in serum. Lipid peroxidation (LPO) in terms of thio barbituric acid reacting substances (TBARS)[11], catalase (CAT)[12], glutathione reductase (GR)[13] and protein[14] were estimated in liver tissue homogenate. A piece of liver tissue was fixed in 10% formalin and was subsequently wax mounted. The Hematoxylein and Eosin stained[15] sections (10 μm thick) were observed under microscope for evaluation of histopathological changes.

The data were subjected to One Way Analysis of Variance (ANOVA) and the significance of the difference between the means of various treatment groups was performed by employing Tukey's multiple comparison test, using SPSS statistical

Table 1—Effect of pretreatment of ethanolic leaf extract of *C. fistula* on various biochemical parameters in liver and serum of rats. [Values are mean ± SE from 6 animals in each group]

Parameters	Group I	Group II	Group III	Group IV	One Way ANOVA (df = 3,20)
Liver Tissue					
LPO [a]	0.032 ± 0.007	0.532 ± 0.01 *	0.036 ± 0.003 **	0.029 ± 0.005 [ns]	F = 1114.06 *P*<0.0005
CAT [b]	22.54 ± 3.44	10.37 ± 1.40 *	21.22 ± 1.46 **	26.26 ± 0.86 [ns]	*F* = 8.880 *P*<0.0005
GR [c]	10.36 ± 0.96	5.38 ± 0.57 *	11.52 ± 0.75 **	11.76 ± 0.62 [ns]	*F* = 13.365 *P*<0.0025
Serum (IU/Lit)					
AST	77.83 ± 4.23	141.28 ± 13.91 *	110.02 ± 6.08 **	93.85 ± 8.45 [ns]	*F* = 7.287 *P*<0.0025
ALT	40.64 ± 7.32	177.52 ± 20.74 *	123.22 ± 15.59 **	45.45 ± 7.90 [ns]	*F* = 17.428 *P*<0.0005
ALP	92.91 ± 11.16	154.92 ± 17.89 *	117.02 ± 6.82 **	112.49 ± 12.63 [ns]	*F* = 5.38 *P*<0.01
LDH	280.34 ± 10.10	432.06 ± 23.50 *	381.53 ± 12.10 **	275.66 ± 22.30 [ns]	*F* = 14.565 *P*<0.0005
γ-GT	88.32 ± 9.42	153.32 ± 10.23 *	112.49 ± 12.63 **	88.88 ± 9.69 [ns]	*F* = 6.624 *P*<0.0025

P values: <0.001; *compared to Group I, **compared to Group II, [ns] = non significant compared to Group I.
[a] : μmole of MDA formed/min/mg protein, [b] : μmole of H_2O_2 utilized/min/mg protein, [c] : μmole of GSH formed/min/mg protein

package (Version 7.5). The values are expressed as mean ± SE and P value < 0.05 was considered significant.

Results and Discussion

CCl_4 alone (Group II) induced hepatocellular damage was evident by an increase in the levels of marker enzymes of liver toxicity i.e., AST (2 folds), ALT (4 folds), ALP, LDH and γ-GT in serum (Table 1), as compared to liquid paraffin alone treated control (Group I). It is postulated that administration of CCl_4 could cause cell lysis, resulting in the release of cytoplasmic enzymes of the liver into the blood circulation, leading to their increase in levels in serum and this property is often implicated to assess the extent of CCl_4 induced hepatocellular damage[16,17]. The observations of the present study are in accordance with these reports. Pretreatment of rats with ethanolic leaf extract (Group III) partially inhibited the increase in the levels of all the above

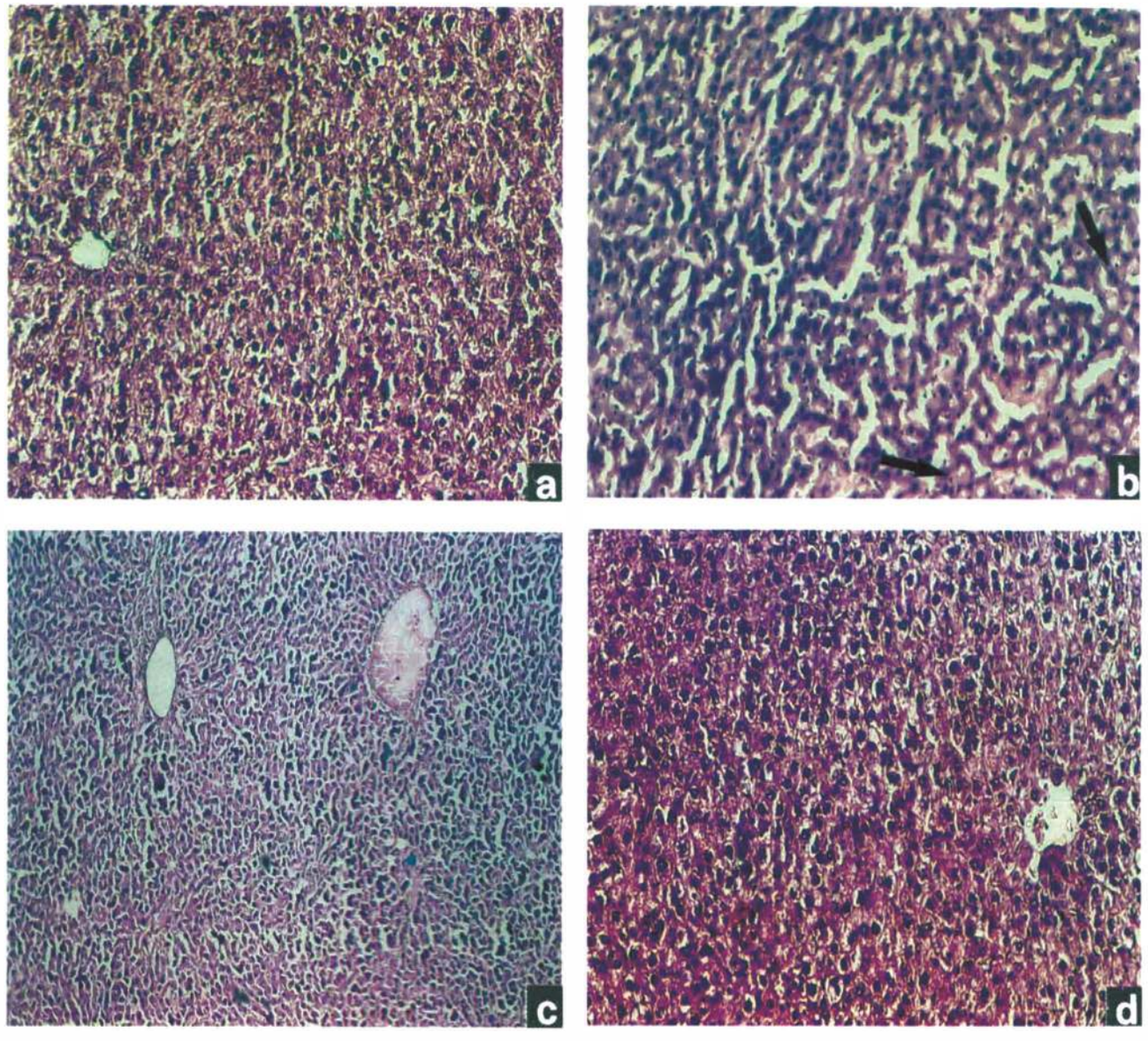

Fig. 1—Histopathology of liver tissue on pretreatment of ethanolic leaf extract of *C. fistula* against CCl_4 induced hepatocellular damage. [(a) (Group I) – normal liver architecture; (b) (Group II) – liver tissue shows hepatocellular necrosis, fatty degeneration and extensive vacuolation; (c) (Group III) – reduction in necrosis and fatty changes; (d) (Group IV) – normal liver architecture, comparable to Group I. H&E. 50×]

marker enzymes of liver toxicity in the serum (Table 1), indicating the hepatoprotective property of the extract.

Hepatocellular membrane damage, consequent to administration of CCl_4 (Group II) was evident by a 16 fold increase in the LPO and 50% reduction in the activities of CAT and GR in the liver tissue (Table 1) as compared to control (Group I). Pretreatment of ethanolic leaf extract for 7 days prior to CCl_4 administration (Group III) completely inhibited the elevated levels of LPO and reversed the decrease in the levels of CAT and GR towards normalcy in the liver tissue. CCl_4 induced liver injury is reported to cause lipid peroxidation[18-20] resulting in membrane damage and the present observations are in accordance with these reports. It is also hypothesized that CCl_4 is metabolically activated by Cytochrome P450 dependent mixed function oxidases to form trichloromethyl free radical ($CCl_3^{\bullet}$) and peroxide radical ($^{\bullet}OOCCl_3$), which are highly reactive and are capable of combining with cellular and membrane lipids in presence of oxygen to induce lipid peroxidation by hydrogen obstruction[21,22]. The complete inhibition of 16 fold increase in LPO and the reversal of 50% reduction in the activities of CAT and GR (Table 1) observed in the present study, clearly demonstrate the strong antioxidant property of ethanolic leaf extract. It is likely that the leaf extract preserves the activity of GR, which maintains the levels of GSH and inhibits LPO by reducing the formation of free radicals derived from CCl_4, thereby accelerating the repair mechanism[23] and thus exhibit significant antioxidant and hepatoprotective effect. Administration of ethanolic leaf extract alone (Group IV), did not produce any alteration in all the parameters studied in the serum and liver tissue and they do not differ from liquid paraffin treated control (Table 1).

Histopathological profiles of the liver from liquid paraffin:CCl_4 treated rats (Group II) showed hepatocellular necrosis, fatty degeneration and extensive vacuolation (Fig. 1b). The protective effect on pretreatment of leaf extract (Group III) is confirmed by significant improvement of hepatocellular architecture over CCl_4 alone treated groups and it is evident by considerable reduction in necrosis and fatty changes (Fig. 1c). The liver sections of rat treated with leaf extract alone (Group IV) showed the presence of normal hepatocellular architecture and absence of necrosis and steatosis (Fig. 1d) and these were comparable with those of liquid paraffin treated control (Fig. 1a).

In conclusion, the present study demonstrates the hepatoprotective and antioxidant properties of ethanolic leaf extract of *C. fistula* during its pretreatment against CCl_4 induced hepatocellular damage. The antioxidant potential and hepatoprotective effect of ethanolic leaf extract could have been brought about by various phytochemical principles i.e., flavonoids, saponins, tannins and alkaloids that are present in the ethanolic leaf extract. In this regard, it is pertinent to point out that flavonoids and tannins have been suggested to act as antioxidants and exert their antioxidant activity by scavenging lipid peroxidation[24]. Thus, the plausible mechanism of the hepatoprotective effect of ethanolic leaf extract that is observed in this study may be due to its antioxidant effect. Further study is warranted to identify and isolate the active biomolecule of ethanolic leaf extract, which offer antioxidant and hepatoprotective properties.

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